1 Introduction

Complex environmental factors are involved in establishment of soil microbial community structure. The abiotic factors like temperature, soil moisture or pH and biotic factors like organic matter, plant cover or soil inhabitants affect major soil processes, which are then reflected in the numbers and taxonomic composition of microorganisms present in a particular soil [1]. Although there is some understanding of the individual effects of some factors on overall microbial activity, limited information is available on how these factors interact to create differences in structure of soil microbial communities [2]. Above that, identification of environmental variables shaping distribution patterns of bacterial communities is a significant first step in delineating functional guilds which are necessary for understanding soil processes [3].

Relationships between plant diversity and soil communities seem critical to ecosystem functioning [4]. Plant species richness determines chemical forms of organic inputs to soil and therefore establish the character of organic matter [5]. Soil microbial communities alter their abundance and taxonomic composition in response to available nutrients [1] and consequently, influence the rate and character of organic matter decomposition [6, 7]. Plants are seldom C limited especially in low fertility habitats such as unimproved pastures, whereas the activity of soil microbes often is [8].

Moisture content is a key factor in soil because it determines the physiological status of bacterial cells [9] and influences the structure of soil microbial communities by effecting the availability and distribution of nutrients and oxygen [10]. Accordingly, in dry soil conditions bacterial communities reacted specifically to the changes in soil moisture regardless of vegetation type, carbon availability or soil temperatures [11]. In situations where soil moisture is not limiting, microbial community was found most affected by plant species [12]. Soil pH has been found to have a strong influence on the composition of soil bacterial communities in many different environments by

Abstract: The effect of environmental factors on bacterial and actinobacterial communities was assessed to predict microbial community structure in natural gradients. Bacterial and actinobacterial communities were studied at four sites differing in vegetation and water regime: creek sediment, wet meadow, dry meadow and deciduous forest located in a shallow valley. The vegetation structure was assessed by phytocoenological relevés. T-RFLP and quantitative PCR were used to determine community composition and abundances. Significant relationships between bacterial community structure and selected soil traits at sites located relatively close to each other (within 200 m) were demonstrated. Both the quantity and structure of bacterial communities were significantly influenced by organic matter content, soil moisture and pH. Bacterial diversity was higher in summer, while that of actinobacteria increased in winter. The Simpson's evenness E was significantly correlated with soil organic matter content. Soil pH had the greatest influence on bacterial community structure showing higher within-site variability in summer than in winter.

Keywords: bacterial communities, actinobacteria, OM quantity and quality, T-RFLP
using various methods over different scales [13, 14]. In particular, diversity of bacterial communities was largely explained by pH, while peak diversity occurred near neutral pH [15].

Actinomycetes belong to the common and often dominating groups of soil bacteria. Yet, the specific distributions of actinobacteria in their natural environment and their connections to soil conditions were rarely addressed. Actinobacteria belong to three phyla, responding strongly to particular site conditions by their proportion within the respective bacterial community [16]. In particular, actinobacteria appeared as the taxonomic group distinguishing among specific soil conditions i.e. drier from wetter, vegetated from unvegetated or dominated with a particular plant species [17, 18]. Actinobacteria were more often associated with high pH [11] and they were typically found in unmanaged ecosystems [19]. They were also described as a group that followed seasonal differences although some studies found them more frequent in summer and others in winter, possibly reacting to soil moisture rather than temperature. [5, 20, 21]. Actinobacteria are efficient at degrading complex plant polymers and affect decomposition processes and organic matter quality [22, 23]. Actinobacteria distribution and diversity are important not only for functioning of soil ecosystems but also because this group represents the most common source of secondary metabolites and new taxa or strains occurring at sites with specific environmental factors may enable discovery of new natural products.

In this study, bacterial communities at four sites with distinct vegetation situated in a creek valley of limestone bedrock were examined. Experimental settings of natural gradients combined with individual sample assessment were chosen to determine variability at our sampling scale. To assess the community composition, a terminal restriction fragment length polymorphism (T-RFLP) analysis and quantification by real time PCR were used. The effect of selected factors demonstrating variability of bacterial and actinobacterial communities at different sites and seasons was assessed to reveal their underlying correlations.

2 Experimental procedures

2.1 Sites

Soil samples were collected at Srbsko, Czech karst region, Czech Republic, in June and December. Four sites, differing in vegetation, mixed deciduous forest (F), dry meadow (DM), wet meadow (WM), and creek sediment (flooded only in spring) (C) were situated along an approximately 500 m transect across a shallow valley. Three samples were collected at each site randomly within an area of 10 m square. The litter (at the forest site) was removed and using a sterile spade and knife, a horizon was cut from the top 15 cm of the soil measuring 10 × 10 cm (approximately 1 kg). Soil was homogenized manually by thorough mixing. Samples were placed in plastic bags, cooled for transport and stored at -20 °C. Sub-samples were analysed for soil particle size, organic matter and nutrient content, and soil pH. The sampling sites represented four distinct vegetation types: Creek sediment (C) enriched with roots of surrounding trees Alnus glutinosa and Acer pseudoplatanus, Wet Meadow (WM) bordering the creek dominated by Agropodium podagraria, Dry Meadow (DM) dominated by Securigera (Coronilla) varia and Bromus erectus and deciduous Forest (F) of Carpinus betulus, Quercus robur and Acer pseudoplatanus (Table S1). The sites also differed in soil characteristics (Table I). In

<table>
<thead>
<tr>
<th></th>
<th>Creek</th>
<th>Wet Meadow</th>
<th>Dry Meadow</th>
<th>Forest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clay (%)</td>
<td>0</td>
<td>11</td>
<td>13.5</td>
<td>22.5</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>12.5</td>
<td>51</td>
<td>56.5</td>
<td>51.5</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>46.9</td>
<td>32</td>
<td>27</td>
<td>21</td>
</tr>
<tr>
<td>Gravel (%)</td>
<td>40.6</td>
<td>6</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>N (%)</td>
<td>0.29</td>
<td>0.27</td>
<td>0.22</td>
<td>0.16</td>
</tr>
<tr>
<td>C:N</td>
<td>22.8</td>
<td>31.1</td>
<td>37.3</td>
<td>56.9</td>
</tr>
<tr>
<td>pH in June (%) ± SD</td>
<td>7.71 ± 0.09</td>
<td>7.53 ± 0.03</td>
<td>7.35 ± 0.04</td>
<td>7.30 ± 0.01</td>
</tr>
<tr>
<td>pH in December (%) ± SD</td>
<td>7.45 ± 0.02</td>
<td>7.51 ± 0.04</td>
<td>7.57 ± 0.04</td>
<td>7.42 ± 0.08</td>
</tr>
<tr>
<td>Moisture in June (%) ± SD</td>
<td>15.9 ± 5.4</td>
<td>23.1 ± 2.5</td>
<td>17.1 ± 3.2</td>
<td>20.4 ± 1.3</td>
</tr>
<tr>
<td>Moisture in December (%) ± SD</td>
<td>19.6 ± 1.6</td>
<td>25.8 ± 3.9</td>
<td>26 ± 10.2</td>
<td>24.3 ± 1.6</td>
</tr>
<tr>
<td>Organic matter in June (%) ± SD</td>
<td>6.8 ± 2.3</td>
<td>8.3 ± 1.8</td>
<td>7.6 ± 0.5</td>
<td>9.3 ± 2.0</td>
</tr>
<tr>
<td>Organic matter in December (%) ± SD</td>
<td>6.4 ± 0.7</td>
<td>8.5 ± 0.5</td>
<td>8.8 ± 4.0</td>
<td>8.9 ± 1.5</td>
</tr>
</tbody>
</table>
particular, there was a significant difference in soil pH between summer and winter seasons at sites C and WM and a significant difference in soil moisture between summer and winter seasons at all sites (Table II, Fig. S1).

**Table 2:** Statistical analyses

a) Multivariate model for T-RFLP correlation with linear variables

<table>
<thead>
<tr>
<th>P-values</th>
<th>Site</th>
<th>Season</th>
<th>Bacteria qPCR</th>
<th>Actinobacteria qPCR</th>
<th>organic matter [%]</th>
<th>pH</th>
<th>moisture</th>
<th>season (for each site)</th>
<th>pH (adjusted to site)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>0.786</td>
<td>0.008***</td>
<td>0.058</td>
<td>0.037*</td>
<td></td>
<td>0.019*</td>
<td>0.024*</td>
<td>0.004**</td>
</tr>
</tbody>
</table>

b) Pearson correlation coefficient’s

<table>
<thead>
<tr>
<th></th>
<th>Bacteria qPCR</th>
<th>Actinobacteria qPCR</th>
<th>organic matter [%]</th>
<th>pH</th>
<th>1/D, bacteria</th>
<th>E, bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria qPCR</td>
<td>0.81</td>
<td>-0.07</td>
<td>-0.12</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Actinobacteria qPCR</td>
<td>0.81</td>
<td>0.09</td>
<td>-0.13</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>% organic matter</td>
<td>-0.07</td>
<td>0.09</td>
<td>-0.32</td>
<td>-0.27</td>
<td>-0.42</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>-0.12</td>
<td>-0.13</td>
<td>-0.32</td>
<td>0.22</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>1/D, bacteria</td>
<td>NA</td>
<td>NA</td>
<td>-0.27</td>
<td>0.22</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>E, bacteria</td>
<td>NA</td>
<td>NA</td>
<td>-0.42</td>
<td>0.18</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

c) P-values

<table>
<thead>
<tr>
<th></th>
<th>Bacteria qPCR</th>
<th>Actinobacteria qPCR</th>
<th>organic matter [%]</th>
<th>pH</th>
<th>1/D, bacteria</th>
<th>E, bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria qPCR</td>
<td>&lt;0.001</td>
<td>0.742</td>
<td>0.563</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Actinobacteria qPCR</td>
<td>&lt;0.001***</td>
<td>0.675</td>
<td>0.548</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>% organic matter</td>
<td>0.74</td>
<td>0.675</td>
<td>0.129</td>
<td>0.294</td>
<td>0.041*</td>
<td>0.389</td>
</tr>
<tr>
<td>pH</td>
<td>0.56</td>
<td>0.548</td>
<td>0.129</td>
<td>0.294</td>
<td>0.389</td>
<td></td>
</tr>
<tr>
<td>1/D, bacteria</td>
<td>NA</td>
<td>NA</td>
<td>0.198</td>
<td>0.294</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>E, bacteria</td>
<td>NA</td>
<td>NA</td>
<td>0.041*</td>
<td>0.389</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

d) ANOVA

<table>
<thead>
<tr>
<th></th>
<th>Bacteria qPCR</th>
<th>Actinobacteria qPCR</th>
<th>pH</th>
<th>moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>site</td>
<td>4.016</td>
<td>0.029*</td>
<td>1.574</td>
<td>0.235</td>
</tr>
<tr>
<td>season</td>
<td>0.04*</td>
<td>0.847</td>
<td>0.383</td>
<td>0.545</td>
</tr>
<tr>
<td>site:season</td>
<td>0.794</td>
<td>0.515</td>
<td>1.153</td>
<td>0.358</td>
</tr>
<tr>
<td>season (winter)</td>
<td>6.237</td>
<td>0.027*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P < 0.05; ** P < 0.01; *** P < 0.001
2.2 Soil analysis

Soil pH was measured in soil water extract: 20 g of soil and 50 ml of water were mixed and left to stand overnight at room temperature. Organic C and total N were determined by high-temperature combustion followed by gas separation in a Vario Max CNS apparatus (Elementar Analysensysteme, Hanau, Germany). Organic matter was estimated by combustion in an oven at 550°C to constant weight. Particle size was determined by standard method at the accredited soil laboratory Geologie und Geotechnica a.s. (Prague, CR; DIN, ISMGE, 1998). Soil DNA was extracted from all replicated samples (total of 24) by the SK method according to [24] as follows: soil (0.5 g) was homogenized in a Mini Bead Beater (BioSpec Products, Bartlesville, OK) for 90 s, at 2500 rpm with 600 µl of extraction buffer (50 mM Na-phosphate buffer, pH 8, 50 mM NaCl, 500 mM Tris-HCl, pH 8, 5% SDS) and 300 µl of phenol/chloroform/isoamyl alcohol (25:24:1) and 0.5 g sterile glass beads (0.25 mg of 0.1 mm and 0.25 mg of 0.5 mm diameter). The homogenate was centrifuged at 16000 × g for 2 min. The supernatant was mixed with the same volume of phenol/chloroform/isoamyl alcohol (25:24:1) and centrifuged at 6000 × g for 5 min. The supernatant was mixed with an equal volume of chloroform/isoamyl alcohol (24:1) and centrifuged at 16000 × g for 5 min. Then, NaCl was added to the supernatant to the final concentration of 1.5 M and CTAB to 1%, and the mixture was incubated at 65°C for 30 min. The solution was cooled, mixed with an equal volume of chloroform/isoamyl alcohol (24:1) and centrifuged at 3400 × g for 20 min. The DNA was precipitated with isopropanol, dissolved in water, incubated with the same volume of 1 M CaCl2, and purified with the GeneClean Turbo DNA kit (Q-biogene, Irvine, CA).

2.3 Terminal restriction fragment length polymorphism analysis and cloning-sequencing

Primers used for amplification of bacterial 16S rRNA genes for T-RFLP were: forward 16Seu 27f (5’-AGAGTTTGATCMTGGCKCAG) [25]; modified [26] labelled with HEX on the 5’ end, and reverse 783r (equimolar mix of 5’CTACCGVGGATCATCTAATCCBG) [27]. For cloning, a non-labelled 27f forward primer combined with PH reverse primer (AAGGAGGTGATCGGCGGCA) [28] for bacteria and act1114r (GAGTTGACCCCGGCRGT) [29] for actinobacteria were used.

All PCRs were performed on T-GRADIENT Thermocycler (Whatman Biometra, Germany). Before adding to PCR mix, 50 ng (in approx. 1-3 ml) of template DNA was preheated with 3 µl BSA (10 mg ml⁻¹) and 6 µl Tris EDTA buffer (10 mM Tris, 0.1 mM EDTA, pH 8) at 90°C for 1 min [19]. PCR amplification consisted of an initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 45 s, annealing at 57°C for 45 s, and extension at 72°C for 90 s with Taq Purple polymerase (Top-Bio, Prague, Czech Republic). PCR products of 16S rRNA using primers 16Seu 27f HEX and 783r were purified by QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and cleaved by Alul for 4 h at 37°C for all samples. In addition, aliquots of PCR products from the DM and F sites were cleaved by HaelI and MspI. After inactivation at 65°C for 20 min the cleaved DNA fragments were purified with Sigma SpinTM Post-Reaction Clean-Up Columns (Sigma-Aldrich, St. Louis, MI). For double-color T-RFLP, purified PCR products of bacterial amplicon obtained with forward primer labelled with HEX and of actinobacterial amplicon labeled with FAM were combined in a proportion 2:1 for simultaneous cleavage by Alul. Details are given in [30]. Fragment analysis was performed at the Genomac International (Prague, Czech Republic, with a 96-capillary sequencer (Applied Biosystems, Foster City, CA). For cloning, PCR were performed with LA polymerase (Top-Bio, Czech Republic) at 68°C for extension. PCR were purified, cloned to pGEM-T Vector (Promega, WI) and sequenced. From winter sampling, 48 bacterial clones (not yet presented in [29]; GenBank Accession Numbers GU194183 to GU194230) and 59 actinobacterial clones (GenBank Accession Numbers GU194231 to GU194289) were sequenced. From summer, 56 bacterial clones (not yet presented in [29]; GenBank Accession Numbers EU715822 to EU715877) and 79 actinobacterial clones previously published [29]; GenBank Accession Numbers EU715878 to EU715976) were used for the analyses.

2.4 Quantitative PCR

The qPCR were run with the forward primer Eub338 (ACTCCTACGAGGACCGACAG) [25] and Actino235 (CCGCGGCTATCGAGCTGTGG) [31] for bacteria and actinobacteria, respectively, both combined with the reverse primer EubS18 (ATTACCGGCGCTGCTGG) [32]. Quantification of the total bacteria and actinobacteria by the 16S rRNA gene copy number in the extracted soil DNA was performed using the iCycler iQ Multicolor Real Time PCR Detection System (BIO-Rad Laboratories, The Netherlands). Amplification was done with SYBR green Super Mix (Bio-Rad Laboratories, The Netherlands). Amplification consisted of 40 cycles, with denaturation (30 s at 95°C), annealing (35 s at 54°C), and elongation (45 s at 72°C), followed by denaturation for 1 min at 95°C and
melting in 80 steps of 10 s from 58°C with 0.5°C increment. Baseline and threshold calculations were performed with the I-Cycler software (version 3.1). The standard curve was calculated based on a cloned PCR product dilution series. The qPCR measurements were done in duplicate. The optimal DNA dilution was first tested and then used for the final qPCR. Since SYBR green might also bind to nonspecific dsDNA, a melting curve was used to assure the specificity of the PCR.

2.5 Statistical analysis

All the statistical computations were conducted within the R statistical computing environment (http://www.r-project.org). Distance-based redundancy analysis, which was implemented by function Adonis of R package Vegan was used to compare scalar variables with distance matrix [33, 34]. Pearson correlation was used to correlate the scalar variables i.e bacterial OTUs with the respective abundances, bacterial diversity indices, soil organic matter content and pH. The Manhattan metric (sum of absolute differences) was used to calculate the distance matrices for terminal restriction fragment analysis. Diversity of bacterial communities was calculated using Simpson’s 1/D and E indices, in which T-RF lengths i.e. ribotypes were used as OTUs and their signal intensities as quantity estimates [35]. Comparison of clone libraries and construction of Venn diagrams was performed in Mothur v.1.6.0 software [36].

3 Results

Significant differences between sites were determined in quantities for bacteria (ANOVA, p = 0.029, Fig. 1a) but not actinobacteria (Fig. 1b). Tukey Honest Significant Differences test showed significant differences between the Creek (C) site and Wet Meadow (WM) site only (p = 0.010). The highest abundances of both bacteria and actinobacteria occurred at the C site. The highest proportion of actinobacteria in the bacterial community was determined at the WM site where they represented 22% of the bacterial community.

Significant differences between bacterial communities were also demonstrated by T-RFLP analysis of bacterial 16S rRNA genes. The community of the C site was separated from the other sites along the first axis and the Forest (F) site was separated from both meadow sites along the second axis. Bacterial communities appeared significantly similar to each other when coming from the same site (p < 0.001, Fig. 2a). Within each site, the communities differed significantly by summer and winter.

Figure 1: Bacteria (a) and Actinobacteria (b) quantities based on the numbers of 16S rRNA gene copies per 1 g soil determined by qPCR. Averages with respective standard deviations, n=3.
seasons (p = 0.004). T-RFLP profiles were significantly correlated to soil moisture, pH, organic matter content and quantity of bacteria, but not actinobacteria. However, after adjustment by site effect, the communities were significantly influenced only by soil pH (p = 0.001, Table II). Diversity expressed as operational taxonomic units was related to quantity determined by qPCR. Simpson’s diversity index 1/D did not differ between sites but the Simpson’s evenness E was significantly highest at the C site (Table S3). The Simpson’s E was significantly correlated with soil organic matter content (Pearson test, p = 0.041, Table II) and soil moisture (Pearson test, p = 0.022, Table II, Fig 3b). This correlation was significantly dependent on the season revealing a tighter relationship in winter when both organic matter content and soil moisture were higher.

Among the 15 T-RF lengths separating the sites, 9 were identified as belonging to actinobacteria (Fig. 2b). The sizes 155, 201, 219, 229, 230, 235, 237, 240, 252 were identified as belonging to actinobacteria as they were also found in the actinobacterial amplicon. T-RF length of 155 was also assigned to Alpha, Beta and Deltaproteobacteria, 201 to Bacilli and Gammaproteobacteria, 240 to Beta and Gammaproteobacteria and Sphingobacteria using the three enzyme restriction (Table S2). Further, 229 T-RF was assigned to Planctomycetes, 230 to Micromonosporaceae, 235 and 237 to Micromonosporaceae and Nocardiaceae using the clone library. T-RFs 201 and 230 were found at

Figure 2: T-RFLP bacterial diversity expressed by PCA. a) Radial arrows show contributions of top 15 T-RFs to site separation, the numbers represent the T-RF lengths (in bp). b) PCA with instrumental variables showing relationships between T-RFLP profiles, bacteria and actinobacteria quantities (qPCR) and environmental factors, soil pH and organic matter. T-RFLP profiles are for sites: Creek (C, square), Wet Meadow (WM, pentagon); Dry Meadow (DM, circle); and Forest (F, triangle), in summer (closed symbols) and winter (open symbols).

Figure 3: a) Correlation between numbers of bacterial OTUs (T-RFs) and abundances based on 16S rRNA gene copy number in DNA extracted from 1 g of soil, as determined by qPCR. b) Correlation between Simpson’s evenness index E and soil organic matter content. Spearman’s rank test was used to calculate the P value.
all sites and both seasons, T-RF length of 219 was present at all sites except for C and WM summer samples and T-RF length of 235 was missing in winter samples of Dry Meadow (DM) and Forest (F) (Table S2).

The F site was studied further by sequencing of a clone library for both bacteria and actinobacteria (Fig. 4a, b). Differences between summer and winter sequences were significant for bacteria (p = 0.013) and actinobacteria (p = 0.009). Chloroflexi were detected only in winter samples. Bacteriodetes were twice, Betaproteobacteria four times and verrucomicrobia eight times more frequent in summer, while Actinobacteria were three times, Alphaproteobacteria three times and Planctomycetes six times more frequent in winter samples. Specific actinobacterial sequencing showed eight suborders of Actinomycetales, which revealed that Micromonosporineae, Propionibacterineae, Micrococcineae and Streptomycineae were equally frequent in summer and winter, while Corynebacterineae and Pseudonocardineae were more frequent in winter. The Venn diagrams showed that the actinobacteria community differed between summer and winter depending on the phylogenetic distance because at 0.03 more specific sequences were determined in summer, however, at 0.13 more specific sequences occurred in winter (Fig. S2).

4 Discussion

In this study, significant relationships between bacterial community structure and selected soil traits were demonstrated at sites located relatively close to each other (within 200 m). The sites differed in vegetation, organic matter content, soil moisture and pH. Significant relationship determined among these variables suggested their connection through decomposition processes because decomposition is influenced by plant species composition, and changed in wet conditions, where it results in decreased soil pH [37].

T-RFLP profiles were significantly correlated to all studied soil variables, however, only correlation to pH was significant after adjusting for site effect. This suggested that soil pH influenced communities at a small scale, while organic matter and soil moisture were shaping communities at a larger scale (i.e., inter-site). In many studies, soil pH acted at a rather large scale and with broad range of pH values [13, 38], however, bacterial community changes due to small differences (only 0.10) in soil pH have been also previously observed [14, 7]. The scale differences might be due to the complexity of soil pH factor i.e. its correlation and functional relatedness to other factors which then determine the scale of changes [39]. Another possible explanation is that small-scale correlation between soil pH and community structure occurs particularly in chalk regions [14; this study] where a qualitative difference in decomposition processes may occur compared to acidic soils. This is because decomposition activities are carried out by many diverse taxonomic groups there but confined to a few taxa in acidic soils [2].

The quantities (qPCR) of both bacteria and actinobacteria differed between sites. The greatest quantity of both groups occurred at the Creek (C) site which had no vegetation but the tree roots, coarse particle structure, and lowest C:N ratio and organic matter content. At other sites, the larger supply of organic carbon was complemented by relatively low N content, which might result in limitation of heterotrophic growth [14]. Also, the availability of carbon might be reduced by clay content at sites WM, DM and F because proportion of non-hydrolyzable C might increase due to adsorption by clay particles [40].

Correlations between quantity and T-RFLP profiles were significant for bacteria but not actinobacteria. This suggests that bacteria react to changes in environmental factors by changing their taxonomic composition together with quantities while actinobacteria community composition remains relatively stable in different quantities possibly due to always performing the same functions. Possibly, it might be also a result of scale because in a large scale observation of six very distant sites (thousands of km) actinobacteria belonged to the more responsive part of the soil bacterial community [16], while in our small scale study, the proportion of actinobacteria in the communities was similar. However, dominating taxa of actinobacteria changed significantly with season at the forest site, for which the clone library was analyzed, suggesting that the small-scale changes occur within the community composition rather than in the quantitative relationships.

Diversity of bacteria expressed as Simpson’s E (evenness) based on T-RFLP profiles was positively correlated with soil moisture and organic matter content which were strongly correlated. This showed that at our sites organic matter and soil moisture stimulated different taxonomic groups evenly in the growth of their populations, therefore not competing for the two resources. This may reflect the previous findings of diversity correlation with overall microbial activity [2] and show that our communities adapt by resource partitioning which leads to increased diversity.
Figure 4: Neighbour joining phyllogram based on Jukes-Cantor distance matrix of 16S rRNA sequences for Bacteria (a) and Actinobacteria (b) bootstrapped over 100 replicates. Vertical lines mark the clusters of respective taxonomic groups (phyla or class for Bacteria and sub-order for Actinobacteria), 9 clusters for Bacteria corresponding to 5 phyla and 4 proteobacterial classes, and 8 clusters for Actinobacteria corresponding to 8 actinobacterial suborders. Filled rectangles present sequences from June, blank rectangles present sequences from December.
Simpson’s E correlations by which relationships are much stronger in winter, can be related to respective variation in organic matter, soil moisture and pH (as demonstrated in Fig. 1 and by correlation of these factors).

The relatively poor bacterial community occurring at the forest site compared to the meadows might be partially explained by the relatively poor chemical composition of organic matter coming from lower plant diversity together with frequency of litter inputs. When inputs are limited to autumn, successions of microbial communities result in time rather than space variability compare to the meadows where the vegetation dies out during the whole growing season [41]. Finally, the organic matter limited environment of the C site supported the highest diversity and evenness agreeing with the finding that starving of organic C leads to community adaptation, not a decrease in diversity [42].

Our results showing higher numbers of Beta-proteobacteria and Verrucomicrobia (based on clones) in summer were similar to those of [21] who also found that these taxa dominate in summer samples. Proportion of actinobacteria in the communities was similar in summer and winter but differed by sites. Using cultivation, actinobacteria diversity was dependent on soil characteristics but abundance was on plant species [43] and they seemed to prefer cold and dry conditions [20]. However, when actinobacteria were studied by pyrosequencing, they changed in relative abundances on a continental scale so they most likely have many different adaptations [15]. In our clones, different actinobacteria occurred in summer and winter samples showing that diversity relationships may differ by taxonomic levels and time points [16]. In winter, unknown actinobacteria were determined in clones from forest soil demonstrating potential bioprospecting strategy.

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